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U.S. EPA HIGH PRODUCTION VOLUME (HPV)
CHEMICALS CHALLENGE PROGRAM

Assessment of Data Availability and Test Plan for Acetyl Tributyl Citrate (ATBC) (CAS RN 77-90-7)

Prepared for:

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CHEMICAL IDENTITY AND USE INFORMATION

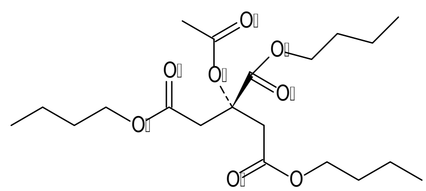
CAS RN

77-90-7

Chemical Name

Acetyl Tributyl Citrate

Structure, Molecular Formula, Molecular Weight



Molecular Formula: $C_{20}H_{34}O_8$
Molecular Weight: 402.5

OTHER CHEMICAL IDENTITY INFORMATION

ATBC
Tributyl O-acetylcitrate
1,2,3-Propanetricarboxylic acid, 2-(acetyloxy)-, tributyl ester
Citric acid, tributyl ester, acetate
Citroflex® A-4

USE PATTERN

Acetyl tributyl citrate (ATBC) is used as a plasticizer with aqueous- and solvent-based polymers, including acrylic, methacrylic, ethyl cellulose, hydroxypropyl methyl cellulose, nitrocellulose, vinyl acetate, vinyl chloride, vinyl pyrrolidone, vinylidene chloride, and urethane polymer systems. ATBC is used in the following applications:

- Medical plastics: Aqueous pharmaceutical coatings; extra-corporeal tubing.
- Food contact products: Food wraps and films; beverage tubing; crown liners; food containers; tinplate lubricant; aluminum foil coatings.
- Cellulosics: Nitrocellulose-based explosives/propellants.
- Other industrial uses: Children's toys; animal ear tags; ink formulations; adhesives; pesticide inerts.

AVAILABLE DATA TO FULFILL HPV SCREENING INFORMATION DATA SET (SIDS) ENDPOINTS

Table 1: Test Plan

ACETYL TRIBUTYL CITRATE CAS RN: 77-90-7		**Information**	**Guideline Study**	**GLP**	**Other Studies Available**	**Estimation Method**	**Acceptable**	**Testing Required**
SIDS Endpoint	**STUDY**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL/CHEMICAL PROPERTIES DATA								
2.1	Melting Point	Y	N	N	Y	Y	Y	N
2.2	Boiling Point	Y	N	N	Y	Y	Y	N
2.4	Vapor Pressure	Y	N	N	Y	Y	Y	N
2.5	Partition Coefficient	Y	N	N	Y	Y	Y	N
2.6	Water Solubility	Y	N	N	Y	Y	Y	N
ENVIRONMENTAL FATE DATA								
3.1.1	Photodegradation	Y	N	N	N	Y	Y	N
3.1.2	Stability in Water	Y	N	N	N	Y	Y	N
3.3.2	Transport and Distribution	Y	N	N	N	Y	Y	N
3.5	Biodegradation	Y	Y	N	Y	Y	Y	N
ECOTOXICITY DATA								
4.1	Acute/Prolonged Toxicity to Fish	Y	Y	Y	Y	Y	Y	N
4.2	Acute Toxicity to Aquatic Invertebrates	Y	Y	Y	N	Y	Y	N
4.3	Toxicity to Aquatic Plants, e.g. Algae	Y	N	N	N	Y	Y	N
HUMAN HEALTH-RELATED DATA								
5.1.1	Acute Toxicity	Y	N	N	Y	N	Y	N
5.4	Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
5.5	Genotoxicity *In Vitro* (Bacterial Test)	Y	Y	Y	Y	N	Y	N
	Genotoxicity *In Vitro or In Vivo* (Chromosome Aberration Tests)	Y	Y	Y	Y	N	Y	N
	Genotoxicity *In Vitro* (Mammalian Cells)	Y	Y	Y	Y	N	Y	N
5.8	Reproductive Toxicity	Y	Y	Y	Y	N	Y	N
5.9	Development Toxicity / Teratogenicity	Y	N	N	Y	N	Y	N

Note: Additional studies include an *in vivo*/*in vitro* unscheduled DNA synthesis study (Appendix, *5.5 Genetic Toxicity In Vitro*), a rat adsorption, metabolism and excretion study and two *in vitro* metabolism studies (Appendix, *5.10 Additional Studies*).

Approach to Evaluate the Database for Acetyl Tributyl Citrate (ATBC)

The following approach was used to obtain and analyze data relevant to the assessment of ATBC.

1. The chemical name and CAS RN of ATBC were provided by Morflex, Inc.
2. Published and unpublished reports were obtained from Morflex, Inc. and other sources; they were organized and reviewed to identify studies that could fulfill SIDS endpoints.
3. Pertinent publicly available databases[1] were searched and all relevant reports were obtained to establish the full extent and nature of the published literature for ATBC.
4. A references database was developed and maintained in order to track reports through the review, assessment and summarization process.
5. Each of the reports obtained was reviewed to determine adequacy according to EPA criteria and reliability according to Klimisch *et al.* (1997).
6. Robust Summaries were prepared for each report with a Klimisch score of 1 or 2, in accordance with the guidelines proposed by the EPA (U.S. EPA, 1999a) for each study type.
7. Physical/chemical properties and environmental fate and ecotoxicity data were estimated by using appropriate Quantitative Structure Activity Relationships (QSARs) (U.S. EPA, 1999b).
8. Fugacity modeling (Level III) was performed to estimate transport and distribution of ATBC into environmental compartments (U.S. EPA, 2000a; Mackay *et al*., 1996a,b).

Use of Structure Activity Relationships for Acetyl Tributyl Citrate (ATBC)

Approaches recommended in the EPA document on the use of structure activity relationships (SAR) in the HPV Chemicals Challenge Program were employed in the assessment of ATBC (U.S. EPA, 1999b). Several SAR-based models, as well as Mackay-type fugacity-based modeling, were employed to support the review and assessment of ATBC. The SAR models for physical properties were used to estimate boiling points, melting points, aqueous solubilities, octanol/water partition coefficients and vapor pressures. Other SAR models were used to estimate hydroxyl radical-mediated atmospheric photo-oxidation and biodegradation potential. SAR models also were used to obtain estimates of acute toxicity to aquatic organisms.

Physical/Chemical Properties QSAR Estimates and Correlation to Reliable Data

Robust Summaries for available reliable studies and QSAR estimates for physical/chemical properties of ATBC are presented in the Appendix.

[1] Databases include ChemIDplus, HSDB (Hazardous Substances Data Bank), IRIS (Integrated Risk Information System), CCRIS (Chemical Carcinogenesis Research Information System), GENE-TOX, EMIC (Environmental Mutagen Information Center), DART/ETIC (Developmental and Reproductive Toxicology and Environmental Teratology Information Center), MEDLINE, TOXLINE, RTECS (Registry of Toxic Effects of Chemical Substances), TSCATS (Toxic Substances Control Act Test Submissions), and IUCLID (International Uniform Chemical Information Database), 1996.

Where possible, the physical/chemical property estimation program EPIWIN version 3.10 was used to derive estimates. QSAR estimates are based on structure and, therefore, can be made only for substances for which a structure can be defined. Since ATBC has a defined structure, a complete set of model data was generated. In general, EPIWIN estimates must be interpreted with a great deal of professional judgment; however, the model estimates for the physical/chemical properties of ATBC are, in most cases, comparable to the available reliable measured data. The available data for physical/chemical properties are summarized below.

Measured data for melting and boiling points were –59°C and 326°C at 760 mm Hg, respectively. The EPIWIN MPBPWIN model-predicted values (U.S. EPA, 2000b) were comparable to the measured data with predicted melting and boiling points of –94.35°C and 410.75°C, respectively. The EPIWIN model also provided an experimental database match for melting point of –80°C, which corroborates the measured and predicted values for melting point.

Vapor pressure was measured as 0.052 mm Hg at 20°C, whereas the EPIWIN MPBPWIN (U.S. EPA, 2000b) model-predicted value was 0.000485 mm Hg at 25°C.

The octanol/water partition coefficient (log K_{ow}) was determined using HPLC to be 4.92 at 22°C and the EPIWIN KOWWIN model-predicted value (U.S. EPA, 2000c) was 4.29 at 25°C.

Measured data and the EPIWIN WSKOWWIN model prediction (U.S. EPA, 2000d) for water solubility were <100 mg/l and 2.045 mg/l at 25°C, respectively. The EPIWIN WSKOWWIN model output also provided an experimental database match of 5 mg/l, which corroborates the predicted value for water solubility.

Environmental Fate and Ecotoxicity QSAR Estimates and Correlation to Reliable Data

Robust Summaries for the reliable studies and QSAR estimates for the environmental fate and effects of ATBC are presented in the Appendix.

The model for atmospheric photodegradation was run according to EPA guidelines. Modeling with EPIWIN AOPWIN (U.S. EPA, 2000e) indicated that ATBC would be expected to degrade rapidly ($t_{½}$ = 0.740 days) upon exposure to ambient light.

The water stability of ATBC was determined using the EPIWIN HYDROWIN model (U.S. EPA, 2000f) and found to be pH dependent. At 25°C and pH 7, the K_b $t_{½}$ = 3.816 days and, at 25°C and pH 8, the K_b $t_{½}$ = 139.394 days. This allows one to conclude that ATBC is moderately stable in water under environmental conditions.

Modeling for environmental transport and distribution (EPIWIN Level III fugacity model, Mackay-type; U.S. EPA, 2000a) predicted distribution to air (mass amount 99%; $t_{½}$ = 17.8 hours) and no appreciable distribution to water, soil or sediment following entry of ATBC into the environment *via* air emissions (1000 kg/hr).

Regarding biodegradation, measured data exist from several studies conducted in various media. The test types and results are summarized in the following table.

Table 2: Summary of Biodegradation Studies

Test Type and Contact Time	Inoculum	Degradation	Results
Standard BOD test 21 days	Unacclimated sewage treatment organisms	Slow rate of degradation under stringent test conditions	100 × (BOD/Chemical Oxygen Demand): Day 5 = 14% Day 21 = 26%
Sewage column degradation 5 hours	Acclimated sewage column sludge	Rapidly biodegradable	> 90% biodegradation in 5 hours
Aerobic biodegradation in soil using a static biometer system 42 days	Soil organisms	Readily biodegradable	Mineralization reached 166.8, 124.4, 97.2, 97.4 and 72.9% as $ThCO_2$ by day 42 in test vessels containing 0.8, 1.6, 3.2, 6.0 and 12.0 mg C/g soil, respectively
Aerobic biodegradation in soil 52 days	Commercial compost seed	Rapidly biodegradable	Mineralization by day 52 reached 128, 125, 90 and 83% $ThCO_2$ for 40, 80, 160 and 300 mg C-treatments, respectively
Ultimate biodegradation in actively aerated compost 45 days	Commercial BOD seed inoculum	Ultimately biodegradable	Mineralization reached 37% $ThCO_2$ in 45 days
Respirometry test in static compost biometer system 28 - 44 days	Compost microorganisms	Readily biodegradable	At 10.8 mg-C/g dry soil, conversion to CO_2 exceeded 60% $ThCO_2$ in three weeks; at 1.9 mg-C/g dry soil, conversion to CO_2 exceeded 60% $ThCO_2$ within four days following the lag period
EPIWIN BIOWIN model (U.S. EPA, 2000g) Not applicable	Not applicable	Fast; readily degradable	$t_{½}$ (water) = 8.67 days $t_{½}$ (soil) = 8.67 days $t_{½}$ (sediment) = 34.67 days

Reliable measured data were available for fish and aquatic invertebrates, and EPIWIN ECOSAR estimates (U.S. EPA, 2000h) for acute fish, daphnid and algal toxicity were modeled. Reliable measured data for acute toxicity to fish were available for mummichogs (*Fundalus heteroclitus*) and bluegill sunfish (*Lepomis macrochirus*) with 96-hour LC_{50} values ranging from 38 – 60 mg/l (nominal concentrations). The 7-day EC_{50} values based on survival and growth of the fathead minnow (*Pimephales promelas*) were 1.9 and 1.4 mg/l, (mean measured test concentrations) respectively. The EPIWIN ECOSAR 96-hour LC_{50} for acute toxicity to the fathead minnow (*Pimephales promelas*) was predicted as 1.669 mg/l. Reliable measured data for acute toxicity to aquatic invertebrates was available for the water flea (*Ceriodaphnia dubia*) with a 48-hour EC_{50} value of 7.82 mg/l (mean measured test concentration). The EPIWIN ECOSAR 48-hour LC_{50} for acute toxicity to the daphnid (*Daphnia magna*) was predicted as 0.704 mg/l. The EPIWIN ECOSAR 96-hour EC_{50} for acute toxicity to green algae (*Selenastrum capricornutum)* was predicted as 0.148 mg/l. Based on the physical/chemical properties of ATBC and the reliable measured 48-hour and 7-day EC_{50} values (as mean measured test concentration) for the water flea (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*), respectively, the predicted EPIWIN ECOSAR values appear to be conservative estimates of the toxicity of ATBC to aquatic species.

Human Health-Related Reliable Data

Robust Summaries for the reliable human health-related studies with ATBC are presented in the Appendix.

Acute oral toxicity data are available for ATBC in rats and cats with LD_{50} values >30 and >50 ml/kg, respectively. Although acute dermal toxicity data are not available, ATBC is predicted to present a very low potential for toxicity *via* the dermal route of exposure because of its extremely high oral LD_{50} and because it is unlikely to be absorbed efficiently through the skin. Acute inhalation toxicity data are not available, but ATBC is predicted to present a very low potential for toxicity *via* the inhalation route exposure because of its low vapor pressure and extremely high oral LD_{50}.

ATBC was studied for repeated dose toxicity in rats, cats and mice in numerous studies ranging in duration from 14 days to 2 years. High doses of ATBC were administered *via* the dietary, oral gavage or intraperitoneal injection routes of exposure. ATBC was shown to possess a low level of subchronic toxicity in rats and cats *via* the oral route of exposure. At very high doses, the few minor changes seen were considered to be a reflection of metabolic adaptation or incidental rather than toxic effects. In two-year oral toxicity studies in rats, ATBC was shown to possess a low level of chronic toxicity potential. No treatment-related toxic effects were reported after two years of daily exposure to high levels of ATBC. Organ specific toxicity was not reported in any of these studies and in all cases the NOAELs are greater than or equal to 100 mg/kg bw/day based on general toxicity associated with high dose exposure regimens.

The numerous testing results for repeated dose toxicity studies summarized in the following table support the conclusion that ATBC has a low potential to cause systemic toxicity.

Table 3: Summary of Repeated Dose Toxicity Studies

Species; Sex	Route	No. of Treatment Groups / No. of Animals per Group	Duration	NOAEL (mg/kg bw/day)
Rat; M & F	Oral (feed)	2 / 4	6 weeks	5%
Rat; M & F	Oral (feed)	2 / 4	8 weeks	10%
Cat; NS	Oral (gavage)	1 / 2	2 months	5250 [a]
Mouse; NS	I.P. injection	1 / 5	14 days	<900
Rat; M & F	Oral (feed)	3 / 10	14 days	<1000
Rat; M & F	Oral (feed)	3 / 40	90 days	300
Rat; NS	Oral (feed)	3 / 20	2 years	100
Rat; M & F	Oral (feed)	3 / 50	*In utero* exposure phase + 90 days	M = 100 F = 300

M = Male; F = Female
NS = Not stated
[a] Assumes a specific gravity of 1.05.

The available battery of six negative *in vitro* genotoxicity assays is adequate to conclude that ATBC does not pose a genotoxicity concern for humans. In addition, ATBC was shown to be negative in an *in vivo*/*in vitro* UDS study, which further indicates an absence of *in vivo* genotoxic potential for ATBC.

The *in vitro* and *in vivo* testing results summarized in the following table support the conclusion that ATBC is neither mutagenic nor genotoxic.

Table 4: *In vitro* and *In vivo* Mutagenicity/Genotoxicity Studies for ATBC

Test System	Test Object	Concentration of Substance	Results
Ames assay (preincubation method)	*S. typhimurium* TA98, TA100, TA1535, TA1537	50 to 5000 µg/plate	Negative[a,b]
Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	333 to 10,000 µg/plate	Negative[a,b]
Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	9 to 495 µg/plate	Negative[b]
In vitro chromosomal aberration assay	Rat lymphocyte cells	4 to 400 µg/ml	Negative[a,b]
In vivo/in vitro unscheduled DNA synthesis	Male rat primary cultures of hepatocytes	Single oral doses of 800 or 2000 mg/kg	Negative
Forward mutation assay	L5178Y (TK+/TK-) mouse lymphoma cells	200 to 480 µg/ml[a] 10 to 230 µg/ml[b]	Negative[a,b]
Forward mutation assay	CHO/HGPRT	25 to 400 µg/ml	Negative[a,b]

[a] With metabolic activation.
[b] Without metabolic activation.

Evaluation of potential for reproductive effects is satisfied for ATBC by a two-generation reproduction study and a 13-week toxicity study with an *in utero e*xposure phase.

In a two-generation reproduction study, no treatment-related clinical observations were noted throughout the study in either F_0 or F_1 parental animals. Body weights of F_0 parents and F_1 females were largely unaffected by treatment with ATBC; however, body weights of the F_1 parental males in the 300 and 1000 mg/kg bw/day groups were consistently lower that controls and appeared to be related to treatment. Body weights of the F_0 females in the 1000 mg/kg bw/day group at the end of pregnancy (gestation days 21 or 22) were significantly lower than control values. Water consumption of the F_0 and F_1 parental animals fed ATBC at a level of 1000 mg/kg bw/day were consistently lower than concurrent controls throughout the study. Mating, gestation and fertility of the F_0 and F_1 generations were unaffected by treatment. There were no abnormalities seen at necropsy that were considered to be treatment-related. The body weights of the pups from the 300 and 1000 mg/kg bw/day dose groups were slightly lower than those of the controls, and slightly higher mortality also was observed in these groups. These effects were considered to be a consequence of the reduced water intakes in the dams at these dose levels rather than a direct effect of ATBC. No other treatment-related effects were observed in the parameters evaluated. Parental and offspring NOAELs both were 100 mg/kg bw/day based on slight body weight effects in the mid- and high-dose groups.

In a 13-week toxicity study with an *in utero e*xposure phase, sensitive reproductive and developmental endpoints were examined. Parental animals were evaluated for reproductive endpoints (mating performance, fertility, gestation length and parturition, litter size, numbers of implantations, survival and growth), and F_1 offspring were evaluated for sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males, sperm assessments and estrous cyclicity), as well as physical appearance, ophthalmologic effects, neurobehavioral effects, growth, food consumption, survival, hematology, blood chemistry, urinalysis, peroxisome proliferation, organ weights, gross pathology and histopathology. Estrous cycles, mating performance, fertility, gestation length and parturition, were all unaffected by treatment. Litter size, survival and growth were similar in all groups and within expected historical control ranges. Although numbers of implantations and litter size at 1000 mg/kg bw/day were marginally lower than concurrent control group levels, they were within the laboratory's historical control ranges. Anogenital distance and sexual maturation in both sexes and retention of areolae in male offspring were unaffected by treatment. There were no adverse effects on sperm motility, counts or morphology. There were no findings at necropsy of parental animals or surplus offspring that were considered to be treatment-related. Parental and offspring NOAELs were 300 and 1000 mg/kg bw/day for reproductive and developmental endpoints, respectively.

ATBC was evaluated for developmental toxicity potential in 12-month studies conducted with rats and mice. Groups of rats and mice were provided feed which contained a milk solution of the test substance (ATBC) at doses of 50 and 250 mg/kg bw/day for 12 months. A third group served as a control. In the ninth month of the study, a cross-mating of the animals was performed, male gonads were evaluated and embryotoxic effects were examined. The following indicators of embryotoxic effects were evaluated: early and late embryonic death (determined by examining the numbers of corpora lutea and implantation sites); and the number of normal, resorptive and deformed tissues. The length of the newborns was measured as was the size and weight of the placenta. Physiological development of the progeny also was evaluated by the following parameters: ear openings, eye openings, appearance of body hair and teeth, behavior and body weight. In both species there were no effects of treatment noted at doses of 50 mg/kg bw/day. ATBC had no significant effects in rats or mice on male gonads, and the spermatogenesis index in animals of the 250 mg/kg bw/day group was similar to controls. Increases in body weight and length of the progeny and placental weight were observed in the 250 mg/kg bw/day dose group. There were no differences between groups in the fertility rate and number of animals born per pregnant female. The physiological development (i.e. eye and ear opening, and body fur and incisor appearance), behavior and body weight of the progeny also were unaffected by treatment. The developmental toxicity NOAEL in both studies was 250 mg/kg bw/day.

Also regarding developmental toxicity, developmental effects were not observed at dose levels as high as 1000 mg/kg bw/day in a two-generation reproduction study nor in a 13-week toxicity study with an *in utero e*xposure phase. ATBC is rapidly and extensively absorbed, and then rapidly metabolized and virtually completely excreted by the rat. The metabolites that have been positively identified in the urine of rats (acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and two isomers of acetyl dibutyl citrate) have been demonstrated to undergo rapid clearance from the body and are not suspected to be developmental toxicants. Also, other ATBC metabolites, acetic acid, citric acid, butyric acid, tributyl citrate and butanol, do not pose a concern for developmental toxicity (see *5.8 Toxicity to Reproduction* and *5.10 Additional Studies*).

SUMMARY OF TEST PLAN

The test plan for physical/chemical properties is summarized in Table 1. Morflex, Inc. contends that the existing measured and modeled data for melting point, boiling point, vapor pressure, octanol/water partition coefficient, and water solubility are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program. No additional data development is suggested for ATBC.

The environmental fate and ecotoxicity test plan is summarized in Table 1. Morflex, Inc. contends that the existing measured and modeled data for photodegradation, stability in water, transport and distribution, biodegradation, acute toxicity to fish, acute toxicity to aquatic invertebrates, and toxicity to aquatic plants are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program. No additional data development is suggested for ATBC.

The human health-related test plan is summarized in Table 1. Morflex, Inc. contends that the existing measured data for acute toxicity, repeated dose toxicity, genotoxicity *in vitro* and *in vivo*, reproductive toxicity, and development toxicity are acceptable to fulfill these endpoints under the U.S. EPA HPV Chemicals Challenge Program, especially when the ATBC rat adsorption, metabolism and excretion study and two *in vitro* metabolism studies also are considered. No additional data development is suggested for ATBC.

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U.S. EPA. (2000c) EPI Suite™, Version 3.10; KOWWIN Program, Version 1.66; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000d) EPI Suite™, Version 3.10; WSKOWWIN Program, Version 1.36; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000e) EPI Suite™, Version 3.10; AOPWIN Program, Version 1.90; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000f) EPI Suite™, Version 3.10; HYDROWIN Program, Version 1.67; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000g) EPI Suite™, Version 3.10; Biodegradation Probability Program (BIOWIN), Version 4.00; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

U.S. EPA. (2000h) EPI Suite™, Version 3.10; ECOSAR Version 0.99g; PC-Computer software developed by ECOSAR Program, Risk Assessment Division (7403), Washington, D.C.